

Drug selection of Leishmania transfectants

1. Recover cells at 25°C for 3-24 hours in 3 ml M199/20% HIFBS.
2. Prepare medium contain different Hygromycin concentration: 0, 5, 10 µg per ml (H0, H5, H10).
3. After recovering the cells, transfer 1 ml of transfectant culture (total 3 ml) into each drug-containing medium (H0, H5, H10).
4. Check cultures daily and record culture turbidity, cell motility and viability.
✂ Viable cells is expected to outcome in 3-4 days, lingering of motile (sluggish) cells in the next 3-7 days. Emergence of very actively motile cells usually swimming on the surface of the culture medium.
5. Passage and cryopreserve transfectants when reaching full turbidity.
6. Grow transfectants in medium with increasing drug concentration (H50, H100, H250 etc).
7. Final grow at H500, G20, T100.